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16. ABSTRACT

Partial and complete life-cycle toxicity tests with fish, involving all developmental stages, have been used extensively in the establishment of water-quality criteria for aquatic life. During extended chronic exposures of fish to selected toxicants, certain developmental stages have frequently shown a greater sensitivity than others. In 56 life-cycle toxicity tests completed during the last decade with 34 organic and inorganic chemicals and four species of fish, the embryo-larval and early juvenile life stages were the most, or among the most, sensitive. Tests with these stages can be used to estimate the maximum acceptable toxicant concentration (MATC) within a factor of two in most cases. Therefore, toxicity tests with these early life stages of fish should be useful in establishing water-quality criteria and in screening large numbers of chemicals.

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Embryo	Organics	Chronic Test	06 T
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Early Life Stages	Chlorine		
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Evaluation of Tests with Early Life Stages of Fish for Predicting Long-Term Toxicity

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Partial and complete life-cycle toxicity tests with fish, involving all developmental stages, have been used extensively in the establishment of water-quality criteria for aquatic life. During extended chronic exposures of fish to selected toxicants, certain developmental stages have frequently shown a greater sensitivity than others. In 56 life-cycle toxicity tests completed during the last decade with 34 organic and inorganic chemicals and four species of fish, the embryo-larval and early juvenile life stages were the most, or among the most, sensitive. Tests with these stages can be used to estimate the maximum acceptable toxicant concentration (MATC) within a factor of two in most cases. Therefore, toxicity tests with these early life stages of fish should be useful in establishing water-quality criteria and in screening large numbers of chemicals.

Key words: fish, embryos, larvae, chronic toxicity, early life stages

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On a fait un usage intensif d'essais de toxicité sur un cycle biologique partiel ou complet avec des poissons, et impliquant tous les stades de développement, afin d'établir des critères de qualité de l'eau pour la vie aquatique. Lors d'expositions chroniques prolongées de poissons à des toxiques choisis, certains stades de développement ont souvent montré une plus grande sensibilité que d'autres. Dans 56 essais de toxicité de tout un cycle biologique effectués durant la dernière décennie avec 34 substances chimiques organiques et inorganiques, et quatre espèces de poissons, les stades les plus sensibles, ou parmi les plus sensibles, ont été le stade embryon-larve et le début du stade juvénile. On peut faire des essais avec ces stades pour estimer la concentration toxique acceptable maximale (CTAM) dans les limites d'un ou de deux facteurs dans la plupart des cas. Les essais de toxicité sur les premiers stades de vie des poissons devraient donc être utiles dans l'établissement de critères de qualité de l'eau et dans l'évaluation d'un grand nombre de substances chimiques.

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The life-cycle toxicity test with fish, involving all developmental stages, was proposed by Mount and Stephan (1967). In these early studies fathead minnows (*Pimephales promelas*) were exposed to a graded series of toxicant concentrations throughout the fish's life cycle, and the effects of the toxicant on survival, growth, and reproduction were measured quantitatively. These quantitative data allowed the determination of a maximum acceptable toxicant concentration (MATC), defined as the hypothetical toxic threshold concentration between the highest concentration tested having no observed effects and the next higher toxicant concentration having significant toxic effects (Mount and Stephan 1967).

Further development of life-cycle toxicity tests with other fish species followed. Eaton (1970) proposed the use of the bluegill (*Lepomis macrochirus*); McKim and Benoit (1971) developed methods for the brook trout (*Salvelinus fontinalis*); and Smith (1973) utilized the flagfish (*Jordanella floridae*) for life-cycle tests. For the last decade, life-cycle toxicity tests have provided the most reliable information on the long-term effects of toxic chemicals on fish and the basis for many of the established water-quality criteria for aquatic life.

During these extended life-cycle tests with several species of fish and a variety of toxicants, certain developmental stages have consistently shown a greater sensitivity than others. The possibility of focusing research efforts on these more sensitive stages holds considerable promise of success in the search for quicker and less costly ways of predicting the chronic toxicity of chemicals to fish. Several investigators have proposed predicting fish chronic toxicity through the use of

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shorter tests with early developmental stages of fish, which, with selected toxicants, were shown to be the most sensitive, or one of the most sensitive, parts of the life cycle (Pickering and Thatcher 1970; Pickering and Gast 1972; McKim et al. 1973; Eaton et al. 1977; McKim et al. 1977). These authors have emphasized that, with only a relatively short exposure of several months of the embryo-larval or early juvenile stages of fish to a toxicant, an estimate of the MATC can be made without carrying out the partial or complete life-cycle test.

This study reviews the available data from life-cycle toxicity tests with fish and evaluates (1) the hypothesis that the embryo-larval and early juvenile stages of development are among the most sensitive stages in life-cycle toxicity tests and (2) the usefulness of embryo-larval and early juvenile toxicity tests in estimating MATCs for fish exposed to individual and complex mixtures of chemicals.

Source of Data

The published and unpublished data from life-cycle toxicity tests with fish were reviewed. Partial and complete life-cycle toxicity studies were included, since a previous evaluation of life-cycle toxicity data by Andrew et al. (1977) indicated that partial life-cycle tests (juvenile through adult spawning to 1 mo after hatching of offspring) gave MATC values similar to complete life-cycle tests (embryos or newly hatched larvae through adult spawning to 1 mo after hatching of offspring). Only tests conducted according to specific methods recommended by the Committee on Aquatic Bioassays of the U.S. Environmental Protection Agency (1972) for fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), brook trout (*Salvelinus fontinalis*), and flagfish (*Jordanella floridae*) were included in this evaluation. Deviations from these methods were allowed only if all developmental stages had been successfully tested and embryo-larval and early juvenile exposures extended to a minimum of 30 days after hatching. The biological effects measured during all embryo-larval and early juvenile fish tests were decreases in hatchability, survival and growth, and increases in deformities.

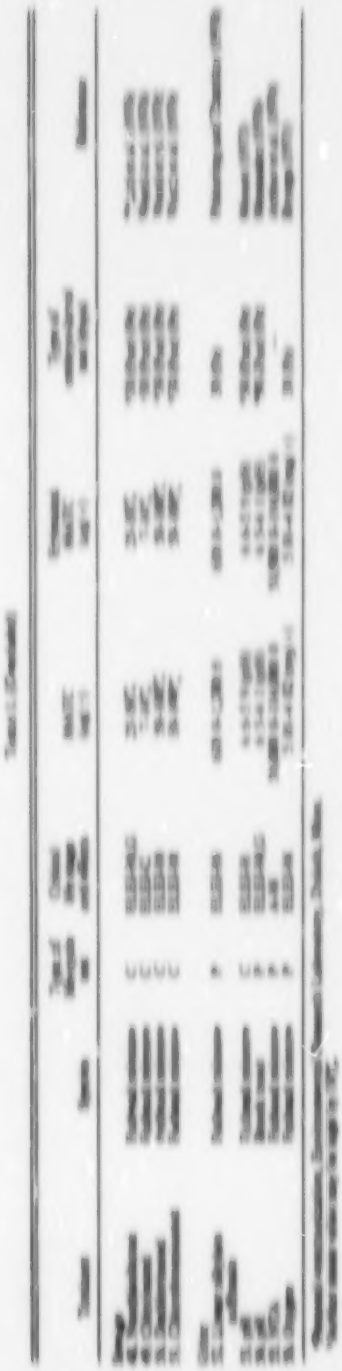
The existing data were also examined to determine if offspring from unexposed parents (P_{UE}) differed in their sensitivity to toxicants from offspring whose parents were exposed (P_E) and offspring whose parents' parents were exposed (P_{EE}).

Results

Data from studies representing 34 toxicants and four fish species are presented in Table 1. In all of the 56 life-cycle tests judged acceptable for evaluation, the embryo-larval or early juvenile estimate of the MATC was within a factor of two of the actual MATC. In 46 (82%) of the tests the embryo-larval or early juvenile estimate of the MATC was essentially identical to the actual MATC. The endpoints in two of these tests (Table 1: test 22, trimetal mixture; 17, zinc) consisted of reduced adhesiveness of the egg and a fragility of the egg membrane, which was considered in the original papers as reduced egg production. Recent life-cycle tests with zinc and fathead minnows (Benoit and Holcombe, personal communication, Environmental Research Laboratory, Duluth, Minn.) showed that the number of eggs spawned was similar in all concentrations if fragile and loose eggs were counted. Therefore, the critical life stage in these earlier papers was considered here as embryo-larval.

Ten life-cycle tests of the total 56 (18%) showed that the embryo-larval or early juvenile estimate of the MATC would have deviated by a factor of two or less from the actual MATC. One of the 10, which initially did not indicate the embryo-larval and early juvenile stages to be the most sensitive, was repeated 3 times, and in each case these early stages were the most sensitive (Table 1: tests 3, 4, 5, and 6, cadmium). In the other nine life-cycle tests (Table 1: test 15, lead; 18 and 19, zinc; 23, methylmercury; 27, chloramine; 33, atrazine; 41, lindane; 46, trifluralin; and 49, Guthion®) the early developmental stages were not among the most sensitive. However, the deviation was again only a factor of two, and in five of these cases (Table 1: tests 15, 18, 19, 23, and 27) other species were tested with the same or very similar compounds and showed the embryo-larval and early juvenile stages were again the most sensitive. The MATCs for a specific toxicant, species, and water combination sometimes vary by a factor of two. For example, flagfish exposed to cadmium in soft water (Table 1: tests 3, 4, 5, and 6) and flagfish exposed to zinc in soft water (Table 1: tests 18 and 19) emphasize this point.

Embryos and larvae from exposed parents were used in all 56 life-cycle toxicity tests evaluated in Table 1, but offspring from both unexposed and exposed parents were used in only 29 tests. In 21 of these tests (75%) no difference in sensitivity between embryos and larvae from previously exposed and unexposed parents was observed (see asterisks, Table 1). The two zinc life-cycle tests mentioned earlier (Table 1: tests 17 and 22) were not included in this evaluation because the embryos (P_{UE}) were exposed after water hardening, and later work (Benoit and Holcombe, personal communication, Environmental Research Laboratory, Duluth, Minnesota) has indicated that zinc



effects show up only when the embryos are exposed before water hardening of the eggs.

In six of the tests (31/9) embryos and larvae from exposed and unexposed parents differed in sensitivity, but never by more than a factor of three. In two of these tests (tests 5 and 6, cadmium) the embryos from exposed parents were more sensitive than those from unexposed parents. In contrast, in a third cadmium test (test 4) run simultaneously with the first two, embryos, larvae, and early juveniles from unexposed and exposed parents did not differ in sensitivity. In other tests with organics (test 38, endrin; 43, malathion), the embryos and larvae of unexposed parents appeared to be more sensitive than those of exposed parents. However, the greater variability in effects between concentrations in the experiment with embryos from exposed parents may have masked the significant effects observed in offspring from unexposed parents. Opposite results were seen with diazinon (tests 37 and 38), where embryos and larvae from unexposed parents seemed to be less sensitive than those from exposed parents. However, in a third diazinon test (test 36) flagfish embryos and larvae from unexposed and exposed parents did not differ in sensitivity.

Embryos and larvae from second-generation exposed parents (Pe_1) were also used in embryo-larval and early juvenile toxicity tests, and their sensitivity was compared with that of their parents (Pe) (Table 1: test 7, cadmium; 10, copper; 14, lead; 20, zinc; 25, methylmercury). Differences in sensitivity were not great enough to alter the MATC values generated by the two groups of offspring.

Thus, in more than half of the 56 life-cycle tests reported in Table 1, the sensitivity of embryo-larval or early juvenile fish from exposed and unexposed parents was usually the same, and in only two tests (37 and 38, diazinon) did it differ by a factor greater than two.

Discussion

Data on 56 life-cycle toxicity tests with four species of fish completed during the last decade with 34 different organic and inorganic chemicals showed the embryo-larval and early juvenile life stages were the most, or among the most, sensitive in life-cycle tests. Toxicity tests with these stages can be used to estimate the MATC within a factor of two in most cases. Eight-two percent of the time the MATC estimated by embryo-larval or early juvenile exposures was identical to the MATC established by the longer, more involved and more costly partial or complete life-cycle

TABLE 2. Estimated maximum acceptable toxicant concentration (MATC) for 12 species of fish established by embryo-larval and early juvenile tests with grouped according to the quality of dilution water used

Water type and test location	Species	Estimated MATC (µg/L)	Type of embryo-larval test	Reference
		Cadmium		
Soft water (44 mg/L as $CaCO_3$) Duluth, Minn.	Flagfish	[illegible]	[illegible]	Spehar (1976)
	Flagfish	[illegible]	[illegible]	[illegible]
	Flagfish	[illegible]	[illegible]	[illegible]
	Flagfish	[illegible]	[illegible]	[illegible]
	Brook trout	[illegible]	[illegible]	[illegible]
	Brook trout	[illegible]	[illegible]	[illegible]
	Brown trout	[illegible]	[illegible]	[illegible]
	Lake trout	[illegible]	[illegible]	[illegible]
	Coho salmon	[illegible]	[illegible]	[illegible]
	Northern pike	[illegible]	[illegible]	[illegible]
	Smallmouth bass	[illegible]	[illegible]	[illegible]
	White sucker	[illegible]	[illegible]	[illegible]
Soft water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Brook trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
Hard water ([illegible] mg/L as $CaCO_3$) Newtown, Ohio	Fathead minnow	[illegible]	[illegible]	Pickering and Gast (1972)
	Bluegill	[illegible]	[illegible]	[illegible]
Hard water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Brook trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
		Chromium		
Soft water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Rainbow trout	[illegible]	[illegible]	[illegible]
	Lake trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
	Bluegill	[illegible]	[illegible]	[illegible]
	White sucker	[illegible]	[illegible]	[illegible]
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Rainbow trout	[illegible]	[illegible]	Benoit (1976)
	Brook trout	[illegible]	[illegible]	Benoit (1976)
		Copper		
Soft water ([illegible] mg/L as $CaCO_3$) Newtown, Ohio	Fathead minnow	10.6–18.4	[illegible]	Mount and Stephan (1969)
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Brook trout	[illegible]	[illegible]	McKim and Benoit (1971)
	Brook trout	[illegible]	[illegible]	McKim and Benoit (1974)
	Bluegill	[illegible]	[illegible]	[illegible]
	Brook trout	[illegible]	[illegible]	[illegible]
	Lake trout	[illegible]	[illegible]	[illegible]
	Brown trout	[illegible]	[illegible]	[illegible]
	Rainbow trout	[illegible]	[illegible]	[illegible]
	White sucker	[illegible]	[illegible]	[illegible]
	Northern pike	[illegible]	[illegible]	[illegible]
Soft water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Brook trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
Hard water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Brook trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
		Lead		
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Brook trout	[illegible]	[illegible]	Holcombe et al. (1976)
	Flagfish	[illegible]	[illegible]	Anderson et al.[1]
Soft water ([illegible] mg/L as $CaCO_3$) Wareham, Mass.	Rainbow trout	[illegible]	[illegible]	[illegible]
	Lake trout	[illegible]	[illegible]	[illegible]
	Channel catfish	[illegible]	[illegible]	[illegible]
	Bluegill	[illegible]	[illegible]	[illegible]
	White sucker	[illegible]	[illegible]	[illegible]
		Zinc		
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Flagfish	[illegible]	[illegible]	Spehar (1976)
	Flagfish	[illegible]	[illegible]	Spehar (1976)
	Brook trout	[illegible]	[illegible]	Holcombe and Benoit[1]
	Fathead minnow	[illegible]	[illegible]	Benoit and Holcombe[1]
		Organic mercury (methylmercuric chloride)		
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Fathead minnow	[illegible]	[illegible]	Mount and Olson[1]
	Flagfish	[illegible]	[illegible]	[illegible]
	Brook trout	[illegible]	[illegible]	McKim et al. (1976)
		Linear alkylate sulfonate (LAS)		
Soft water ([illegible] mg/L as $CaCO_3$) Duluth, Minn.	Fathead minnow	[illegible]	[illegible]	[illegible]
	Northern pike	[illegible]	[illegible]	[illegible]
	Smallmouth bass	[illegible]	[illegible]	[illegible]
	White sucker	[illegible]	[illegible]	[illegible]

[1]Personal communication, Environmental Research Laboratory, Duluth, Minn.

toxicity test. Water quality, species, type of toxicant, and parental exposure had little effect on these conclusions.

If the above is true for individual toxicants, it should also be true of toxicant mixtures or complex effluents. Little information is available on life-cycle toxicity tests with complex effluents or mixtures, but the work cited in Table 1 on sewage (tests 49–51) and metal mixtures (test 33) supports this assumption to some degree.

Data on the sensitivity of various species to a single toxicant in the same water have been summarized in Table 2. With some toxicants species differences in sensitivity were not very great, yet with other toxicants sensitivity varied considerably among species. Thus the most important species or the most sensitive, if known, should be tested in any given situation. For screening large numbers of chemicals the use of one or two consistently sensitive species (e.g. fathead minnows) would probably suffice until more is known about species sensitivity to specific types or classes of compounds.

An estimate of the MATC based on tests with early life stages could be used with the 96-h LC50 to produce an application factor (AF = estimated MATC/LC50), similar to that defined by Mount and Stephan (1967) (AF = MATC/LC50) for use in extrapolating to other situations. However, the validity of such a relationship would have to be examined (Andrew et al. 1977).

Laboratory methods are now available for embryo-larval tests that would allow the relatively rapid screening of chemicals with selected species. Further development of these tests for the laboratory and for on-site (field) testing is under way, with the major emphasis on reducing the duration of exposure.

Mount et al. (1976) described the need in mammalian toxicology for more rapid, less costly tests to screen out potentially carcinogenic and toxic chemicals from the ever-expanding list of potentially useful chemicals. Faster, less costly testing procedures are also of extreme importance in aquatic toxicology, and in the USA this is especially so with the implementation of the Federal Environmental Pesticide Control Act of 1972 and the Toxic Substances Control Act of 1976, both of which will require prescreening by industry of some chemicals before commercial production. The use of embryo-larval fish toxicity tests would make possible the screening of large numbers of chemicals rapidly at a far lower cost per test than a partial or complete life-cycle toxicity test.

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